Attachment 2a. First Analysis QuEChERSs Extraction Method

Water Samples:

1) Transfer 10 ml or 10 g of water samples into 50 mL plastic centrifuge tubes.

2) Prepare procedural blank (Blank) and laboratory control spike (LCS) samples by transferring 10 ml (or 10 g) reagent water into 50 ml of plastic tubes.

3) Prepare a matrix spike (MS) by transferring 10 ml (or 10 g) from one the samples into a 50 ml plastic tube.

4) Spike the LBS and MS sample with fortification standard.

5) Add 15 ml acetonitrile and 0.15 ml of acetic acid to each sample. Cap the sample tubes and shake.

6) Add premixed MgSO4/NaCl salt mixture (6 g anhydrous magnesium sulfate and 1.5 g sodium chloride) and shake immediately. Make sure the salt clumps are broken apart.

7) Place the samples on a mechanical shaker and shake vigorously for 30 min.

8) Centrifuge at 4,000 RCF for 5 minutes.

9) Transfer two aliquots of at least 4 mL of the supernatant into separate test tubes marked for either GC or LC analysis for each sample and evaporate until near dryness under a stream of N_2 on a water bath at 50^{o}C.

11) **For LC/MSMS analysis**, add LC internal standard solution to one aliquots of the samples. Add 0.5 ml of methanol/water (70/30) to each sample tube and vortex.

12) Transfer the samples to LC vials for LC/MSMS analysis.

13) **For GC/MSMS analysis,** add GC internal standard solution to the second aliquots of the samples. Add 0.5 ml of acetone to each sample tube and vortex.

14) Transfer the samples to GC vial for GC/MSMS analysis.

Soil/sludge Samples:

1) Weigh 10 g of samples into 50 ml plastic centrifuge tubes.

2) Prepare procedural blank (Blank) and laboratory control spike (LCS) samples by weighing 10 g of clean sand (for blank) and field blank soil (for LCS) into 50 ml plastic tubes.

3) Prepare a matrix spike (MS) by weighing 10 g of one the samples into a 50 ml plastic tube.

4) Spike the LCS and MS samples with fortification standard solution.

5) Add 15 ml of acetonitrile and 0.15 ml of acetic acid to each sample.

6) Add 3-4 ml of reagent water to the samples if any of the samples is dry or has low moisture.

7) Cap the sample tubes and shake for 1 min.

8) Add premixed MgSO4/NaCl salt mixture (6 g anhydrous magnesium sulfate and 1.5 g sodium chloride) and shake immediately. Make sure the sample clumps are broken apart.

9) Place the samples on a mechanical shaker and shake vigorously for 30 min.

10) Centrifuge at 4,000 RCF for 5 minutes.

11) Transfer at least 5 mL acetonitrile extract into two separate cleanup tubes that contained dispersive SPE media ($MgSO_4$/PSA/C_{18}) for each sample. Shake and centrifuge.

12) Transfer two aliquots of at least 4 mL of the supernatant into separate test tubes marked for either GC or LC analysis for each sample and evaporate until near dryness under a stream of N_2 on a water bath at 50°C.

13) **For LC/MSMS analysis** Add 0.5 ml of methanol/water (70/30) to each sample LC marked tube and vortex.

14) Transfer 250 µL of samples to LC push-filter vials (0.2 µm filter), add LC internal standard, and push solution through for LC/MSMS analysis.

15) **For GC/MSMS analysis,** Add 0.5 ml of acetone to each sample tube and vortex.

16) Transfer 250 µL of samples to LC push-filter vials (0.2 µm filter), add GC internal standard, and push solution through for GC/MSMS analysis.

LC Analysis:

Agilent 1200 LC/6460 triple quad MS under MRM mode.

Column:	Agilent Zorbax C18 column 100 mm x 2.1 mm, 1.7 um
Mobile phases:	A: Aqueous 5 mM ammonium formate with 0.1% formic acid. B: Methanol with 5 mM ammonium formate/0.1% formic acid.

The LC will be operated with a gradient from 95% A and 95% B in 12 min and holding at 95% B for 5 min before returning to 95% A.

GC Analysis:

Agilent 6870 GC/7000 triple quad MS operated under MRM mode

Column:	30 m x 0.25 mm (i.d.) DB-5ms, gas flow rate of 1.2 ml/min.
Oven temperature:	Hold at 70ºC for 1 min, raise to 150ºC at a rate of 15ºC/min, then to 220oC at a rate of 6ºC/min, hold for 5 min and then raise to 300oC at a rate of 8ºC/min, hold for 10 min.

Attachment 2b. Second Analysis QuEChERSs Extraction Method

Water Samples:

10) Transfer 10 ml or 10 g of water samples into 50 mL plastic centrifuge tubes.

11) Prepare procedural blank (Blank) and laboratory control spike (LCS) samples by transferring 10 ml (or 10 g) reagent water into 50 ml of plastic tubes.

12) Prepare a matrix spike (MS) by transferring 10 ml (or 10 g) from one the samples into a 50 ml plastic tube.

13) Spike the LBS and MS sample with fortification standard.

14) Add 15 ml acetonitrile and 0.15 ml of acetic acid to each sample. Cap the sample tubes and shake.

15) Add premixed MgSO4/NaCl salt mixture (6 g anhydrous magnesium sulfate and 1.5 g sodium chloride) and shake immediately. Make sure the salt clumps are broken apart.

16) Place the samples on a mechanical shaker and shake vigorously for 30 min.

17) Centrifuge at 4,000 RCF for 5 minutes.

18) Transfer at least 10 ml from top layer (acetonitrile) into a separate test tube containing premixed SPE media (900 mg $MgSO_4$/300 mg PSA/150 mg graphite) for each sample, followed by a round of shaking and centrifugation at the parameters above

19) Transfer two aliquots of at least 4 mL of the supernatant into separate test tubes marked for either GC or LC analysis for each sample and evaporate until near dryness under a stream of N_2 on a water bath at 50^{o}C.

11) **For LC/MSMS analysis**, add LC internal standard solution to one aliquots of the samples. Add 0.5 ml of methanol/water (70/30) to each sample tube and vortex.

12) Transfer the samples to LC vials for LC/MSMS analysis.

13) **For GC/MSMS analysis,** add GC internal standard solution to the second aliquots of the samples. Add 0.5 ml of acetone to each sample tube and vortex.

14) Transfer the samples to GC vial for GC/MSMS analysis.

Soil/sludge Samples:

1) Weigh 10 g of samples into 50 ml plastic centrifuge tubes.

2) Prepare procedural blank (Blank) and laboratory control spike (LCS) samples by weighing 10 g of clean sand (for blank) and field blank soil (for LCS) into 50 ml plastic tubes.

3) Prepare a matrix spike (MS) by weighing 10 g of one the samples into a 50 ml plastic tube.

4) Spike the LCS and MS samples with fortification standard solution.

17) Add 15 ml of acetonitrile and 0.15 ml of acetic acid to each sample.

18) Add 3-4 ml of reagent water to the samples if any of the samples is dry or has low moisture.

19) Cap the sample tubes and shake for 1 min.

20) Add premixed MgSO4/NaCl salt mixture (6 g anhydrous magnesium sulfate and 1.5 g sodium chloride) and shake immediately. Make sure the sample clumps are broken apart.

21) Place the samples on a mechanical shaker and shake vigorously for 30 min.

22) Centrifuge at 4,000 RCF for 5 minutes.

23) Transfer at least 10 mL acetonitrile extract into two separate cleanup tubes that contained dispersive SPE media ($MgSO_4$/PSA/C_{18}) for each sample. Shake and centrifuge.

24) Transfer at least 10 ml from top layer (acetonitrile) into a separate test tube containing premixed SPE media (900 mg $MgSO_4$/300 mg PSA/150 mg graphite) for each sample, followed by a round of shaking and centrifugation at the parameters above

25) Transfer two aliquots of at least 4 mL of the supernatant into separate test tubes marked for either GC or LC analysis for each sample and evaporate until near dryness under a stream of N_2 on a water bath at 50°C.

26) **For LC/MSMS analysis**, add LC internal standard solution to one aliquots of the samples. Add 0.5 ml of methanol/water (70/30) to each sample tube and vortex.

27) Transfer the samples to LC vials for LC/MSMS analysis.

28) **For GC/MSMS analysis,** add GC internal standard solution to the second aliquots of the samples. Add 0.5 ml of acetone to each sample tube and vortex.

29) Transfer the samples to GC vial for GC/MSMS analysis.

LC Analysis for Most Analytes:

Agilent 1200 LC/6460 triple quad MS under MRM mode.

Column:	Agilent Zorbax C18 column 100 mm x 2.1 mm, 1.7 um
Mobile phases:	A: Aqueous 5 mM ammonium formate with 0.1% formic acid. B: Methanol with 5 mM ammonium formate/0.1% formic acid.

The LC will be operated with a gradient from 95% A and 95% B in 12 min and holding at 95% B for 5 min before returning to 95% A.

LC Analysis for Analytes present in Large Quantities/Analyte Carry-over:

Agilent 1200 LC/6460 triple quad MS under MRM mode.

Column:	Agilent Zorbax C8 column
Mobile phases:	A: 0.1% Formic Acid in Water. B: Acetonitrile.

The LC will be operated with a gradient from 95% A and 95% B in 12 min and holding at 95% B for 5 min before returning to 95% A.

GC Analysis:

Agilent 6870 GC/7000 triple quad MS operated under MRM mode

Column:	30 m x 0.25 mm (i.d.) DB-5ms, gas flow rate of 1.2 ml/min.
Oven temperature:	Hold at 70ºC for 1 min, raise to 150ºC at a rate of 15ºC/min, then to 220oC at a rate of 6ºC/min, hold for 5 min and then raise to 300oC at a rate of 8ºC/min, hold for 10 min.

Attachment 2c. Glyphosate and Glufosonate Extraction Method

For Water Samples:

1. Measure approximately 5 g of water sample into a 50 mL polypropylene test tube.

2. Prepare a Laboratory Control Blank (PB) and a Laboratory Control Spike (LCS) by measuring 5 g of DI water into a 50 mL polypropylene tube. Also prepare a field blank spike (FBS) by weighing 5 g of a field water blank into a 50 mL tube.

3. Spike LCS and FBS with a fortification standard solution.

4. To all samples and spikes add mass-labelled internal standard solution.

5. Add 58.5 µL of concentrated (>85%) phosphoric acid to every test tube.

6. Shake samples on digital pulse mixer for 1.5 hours, followed by sonication for 15 minutes.

7. Using a disposable transfer pipette, transfer about 1.5 mL of the extractant into a 6 mL Luer-lock syringe, with a 0.45µm glass or nylon filter attached to the end, and a Grace Maxi-Clean IC-RP SPE (or equivalent) cartridge attached after that.

8. Push the liquid through the syringe into an LC autosampler vial at the rate of 2-3 drops per second.

For Soil Samples:

1. Measure approximately 5 g of soil sample into a 50 mL polypropylene test tube

2. Prepare a Laboratory Control Blank (PB) and a Laboratory Control Spike (LCS) by measuring 5 g of DI water into a 50 mL polypropylene tube. Also prepare a field blank spike (FBS) by weighing 5 g of a field soil blank into a 50 mL tube

3. Spike LCS and FBS with a fortification standard solution

4. To all samples and spikes add mass-labelled internal standard solution

5. Add 25 mL of 0.1 M NaOH solution

6. Shake samples on digital pulse mixer for 1.5 hours, followed by sonication for 15 minutes.

7. Using a disposable transfer pipette, transfer about 1.5 mL of the extractant into a 6 mL Luer-lock syringe, with a 0.45µm glass or nylon filter attached to the end, and a Grace Maxi-Clean IC-RP SPE (or equivalent) cartridge attached after that.

8. Push the liquid through the syringe into a 15 mL polypropylene tube at the rate of 2-3 drops per second.

9. Combine 966 µL of the filtrate with 34 µL of 30% H_3PO_4 solution into an LC autosampler vial.

LC Analysis:

Agilent 1200 LC/6460 triple quad MS under MRM mode.

Column:	Bio-Rad Micro-Guard Cation H Cartridge
Mobile phases:	A: 0.1% Acetic Acid
	B: Acetonitrile

The LC will be operated with an isocratic flow of 80:20 A:B at 0.5 mL/min